Flowcytometry or Luminex Crossmatch Comparison and Limitations

Mahendra Mishra | Aarti Khanna Nagpal | Sarjana Dutt

Pathkind Diagnostics Pvt. Ltd.
Gurugram India

ABSTRACT

Aim To evaluate Luminex crossmatch (LXM) for pretransplant work up and compare it with Flow crossmatch (FCXM) and solid – phase assays (SPA) in a limited resources setting.

Methods 95 living donor recipients were tested by LXM & FCXM. SPA was done on 50 samples according to the protocol mentioned in the product insert using kits from Werfen, India, and accrued data was analysed on a Luminex 200 analyser. Three colour FCXM was done on Navios Beckman Coulter Flowcytometer. Patients were followed up for 3-6 months after transplantation.

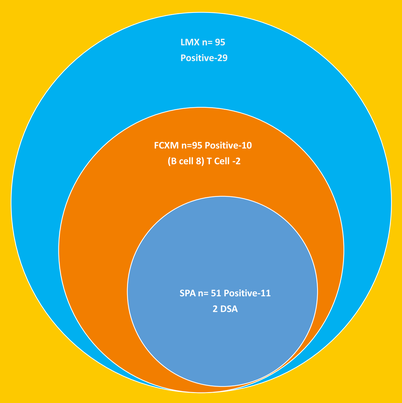
Results

As mentioned in Poster.

High background was seen in 26 (27%) samples that were processed for LXM, but it affected the results only in two samples. None of the patients with a positive LXM had adverse impact on transplanted kidney.

Conclusions Limitations of Luminex crossmatch include high incidence of false positive results especially false positive Class II IgG and high background which mandate additional testing. FCXM was superior to LXM for pretransplant work up and adding the latter was of no additional value.

Antibody assays: A Pilot Study



Contact:

Dr (Col) Mahendra N Mishra
Pathkind Diagnostics Pvt. Ltd,
Gurugram, India
Mahendra.Narain@pathkindlabs.com
+917838101202

INTRODUCTION

Introduction Luminex crossmatch (LXM) is often used in India either alone or in combination with Flow crossmatch (FCXM) & CDC crossmatch for pretransplant evaluation of living donor recipients due to its low cost. B and T Cell FCXM and LXM were performed on 95 samples for living donor renal transplants. Solid phase assays (SPA) were performed for 50 (52.5%) samples. Four aberrant results are also discussed

METHODS AND MATERIALS

Both FCXM and LXM were performed on 95 samples Analysis was done on Navios Beckman Coulter (1024 channel) and Luminex 200 respectively

- Pooled bead /phenotype or single antigen bead assay carried out on 50 samples with Werfen (India) kits according to the protocol mentioned in the product insert. This was taken as **reference result** for both crossmatches

RESULTS

False positive LXM Class II (17), Class I (1) and both(4)

- Both LXM and FCXM false positive (1), LXM more accurate for 2
- Even with DSA MFI > 15000 T Cell FCXM negative for 3 samples
- Isolated T Cell crossmatch positive with MCS α SAB MFI for 3 samples
- Donor A*24:02L, SAB MFI 2850, FCXM (B+) done twice, LXM CI 1 & II MFI > 2500, Score 0 (negative)
- Unsensitized male with Screen and SAB negative had B Cell FCXM and LXM CI2 (MFI 612) positive

Table 1. Comparison of FCXM & LXM Results

Tests	Numbers (n)	SPA (n)	High BG LXM
FCXM & LXM -	63	27	16
FCXM- , LXM +	24	17	7
FCXM +, LXM -	2	2	1
FCXM & LXM +	5	3	2

Table 2. SPA Data

SPA Assay	(n)
Pooled	23
Phenotype	19*
SAB	8**

Figure 1. Navios Flowcytometer

Figure 2. Luminex 200.

RESULTS

Case #1

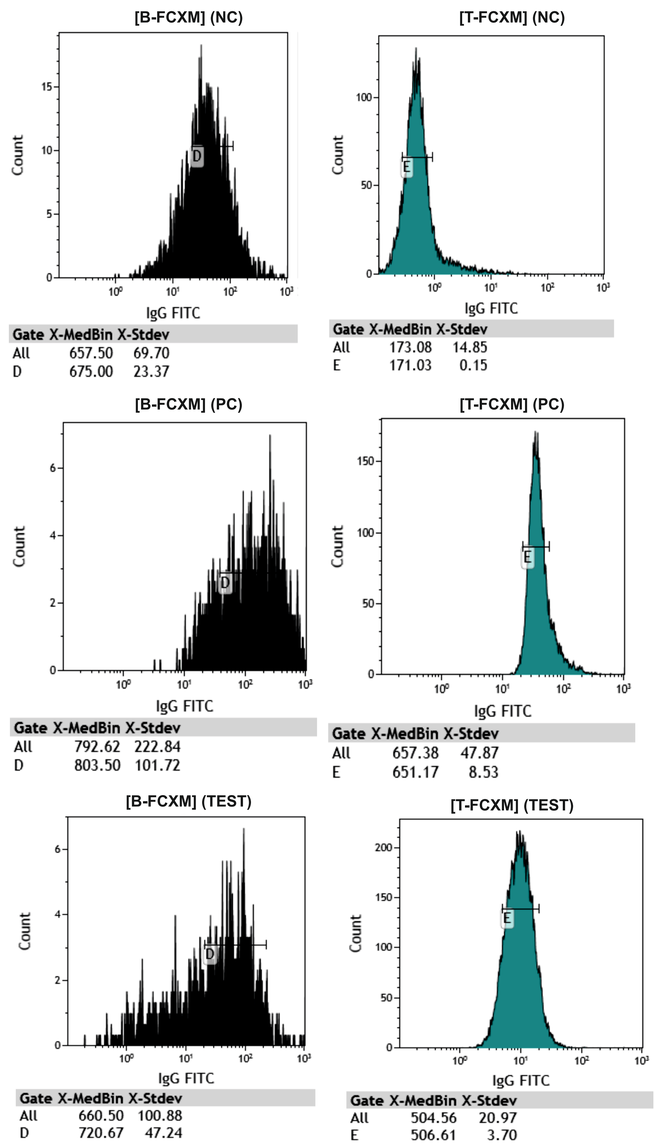
51 years female , ESRD cause unknown, G3P2A1
Previous transplant May 2007 (1 /12 match), No transfusions
Dialysis vintage: Jun 2023, Donor typing NA

Table 4. HLA Typing Case #1 And Donors

Individual	HLA-A*	HLA- B*	HLA- C*	HLA-DRB1*	HLA□DQB1	-DPB1
Patient	11:01,11:01	52:01,52:01	12:02, 14:03	14:04,14:04	05:03.05:03	02:01,04:01
Donor 1	03:01,11:01	13*01,52:01	04:03,12:02	12:02,15:02	03:01,06:01	21:01,1088:01
Donor 2	01:01,02:01	13*01,40:06	01:02. 01:02	15:01,15:01	06:01,06:01	01:01,02:01
Donor 3	01:01,01:01	37:01,37:01	06:02,06:02	01:01,10:01	05:01,05:01	02:01,09:01

Table 5. Summary of FCXM, LXM & SAB Assay

	FCXM B/T	LXM Class I / II (MFI	SAB (DSA)MFI
D1	-29 / 17	409 / 689 (0)	1218 (DQ7)
D2	+38 /267	963 (0)/ 522 (0)	17601 (A1,A2)
D3	-45 / 335	1349(3)/ 426 (0)	30544 (A1, B37)
Reference	>90(T) />60 BMCS	MFI> 500 + score >1	> 1000



DISCUSSION

LXM is used in India either alone or in addition to FCXM

High incidence of background and false positive especially Class II (reported previously also)

Both tests discordant in 25 (26 %) of which LXM more accurate in 4 (true negative -3 , true positive -1)

Reduced sensitivity of BFCXM (?) causing isolated T Cell FCXM positive with channel shift α SAB MFI and negative results even with MFI >15000 (HLA-B*51) Table 6

LXM must be combined with pooled bead assay to identify false positive results and resolve high BG issues

Data derived from routine patent evaluation in a Standalone commercial laboratory; repeat samples – not possible

Unexplained findings:

Negative B – Cell FCXM with defined DSA by SAB analysis against HLA-DRB1, HLA-A & B alleles

FCXM positive even with low MFI DQB1 DSA

Positive FCXM with MFI 2185 against HLA-*24:02L

Pooled bead / Phenotype assay adequate negative marker but SAB mandatory for confirmation

Limitations : Donor specific antibodies, not identified by SAB for . High background could influence LXM results

Date	DONOR	DSA (MFI)	FCXM
12/6	Mother	B*52:01 (16273)	-20 /+21
26/6	Mother	B*52:01 (16273)	+38 /90 (T)
01/7	Father	B*51:01 (19238)	112/37 (B)
12/7	Uncle	B*40:06 (10230)	-100 /+3

CONCLUSIONS

Luminex crossmatch was discordant with respect to Flow crossmatch in >26 % cases and high background was present in up to 25% of samples which was not seen when same sample was evaluated for other SPA

It was of no additional value when added to FCXM for pretransplant work up

Solid phase assay helped is identifying false positive crossmatches

REFERENCES

1. Ameur RF et. al Scand J Immunol 2023; 98(1):e13279. doi: 10.1111/sji.13279. Epub 2023 May 14.
2. Mishra MN. et al. Int J Transplant Res Med 2019; 5:039.

Acknowledgment: EFI for providing travel support